MAD Microfocus Macromolecular Crystallography at Diamond Light Source

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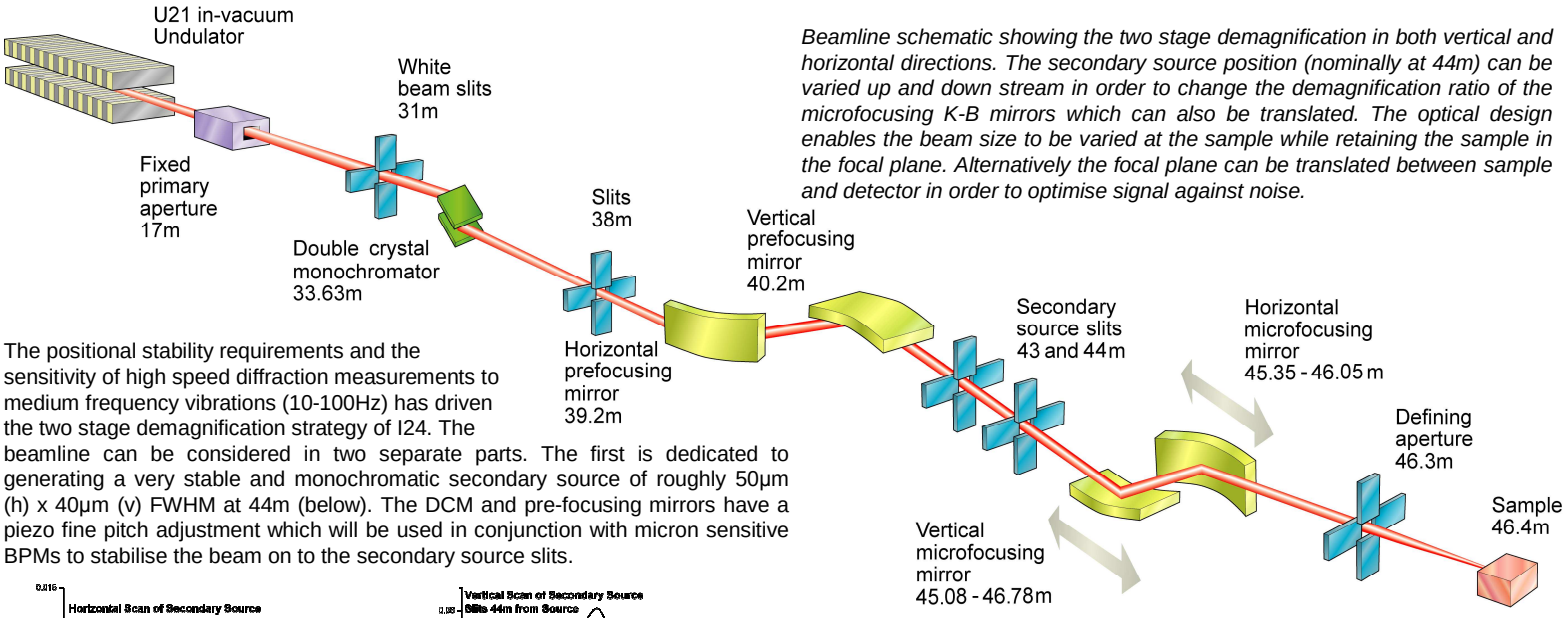
Introduction

Increasingly, a critical restraint on the application of X-ray diffraction has been the difficulty in obtaining crystals of sufficient size (50-100 microns) and order to generate the high quality diffraction patterns required for the detailed analysis. This is notably true for membrane proteins and multi-protein complexes, which play a central role in cellular operation. A consequence of the drive to increase the rate of crystal automation of protein expression, screening and crystallisation is the growing dependence on small amounts of material for the structural determination. A solution to this problem is to reduce the beam size and preferentially illuminate smaller crystals or more perfect regions.

Key Features

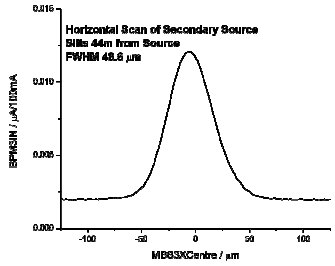
Diamond : 3GeV, 300 mA, 2.7 nm.rad
Source : 2m long 21mm period undulator (> 5mm gap)
Canted ID angle : 1.5 mrad outboard
Energy range : 6.5-25 keV
Energy resolution : ~2 × 10^{-4} (ΔE/E of Si 111)
Flux @ 12 keV at sample : > 10^{12} ph/s into focal spot
Beam size at sample (focused) : < 5μm (h) × 5μm (v), FWHM
Beam diverg. at sample (h x v) : <2.0mrad × 0.3 mrad
Harmonic rejection : 10^{-4}

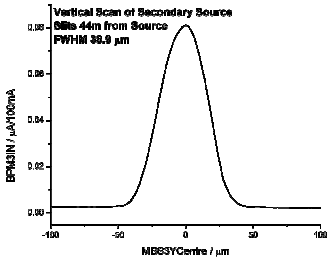
Beamline design



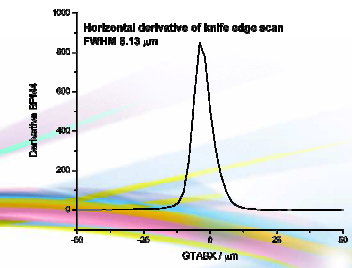
Beamline schematic showing the two stage demagnification in both vertical and horizontal directions. The secondary source position (nominally at 44m) can be varied up and down stream in order to change the demagnification ratio of the microfocusing K-B mirrors which can also be translated. The optical design enables the beam size to be varied at the sample while retaining the sample in the focal plane. Alternatively the focal plane can be translated between sample and detector in order to optimise signal against noise.

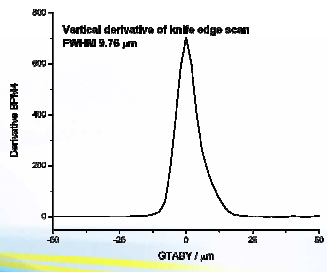
The positional stability requirements and the sensitivity of high speed diffraction measurements to medium frequency vibrations (10-100Hz) has driven the two stage demagnification strategy of I24. The beamline can be considered in two separate parts. The first is dedicated to generating a very stable and monochromatic secondary source of roughly 50μm (h) x 40μm (v) FWHM at 44m (below). The DCM and pre-focusing mirrors have a piezo fine pitch adjustment which will be used in conjunction with micron sensitive BPMs to stabilise the beam on to the secondary source slits.





The second stage of I24 is an ultra-stable microfocusing stage consisting of K-B mirrors, beam conditioning and diagnostics unit, sample stage and goniometer mounted on a common support structure. No feedback will be used within this shorter stage since residual instabilities in the secondary source will be further demagnified to a negligible level.
During the commissioning phase, users have access to two optical arrangments, ‘mini’ and ‘maxi’. The mini configuration results in a beam of ~ 9μm (h) x 9μm (v) FWHM at the sample (below). The maxi beam is roughly 40μm (h) x 50μm (v) FWHM at the sample and is mostly commonly used during the grid scan (details to right).

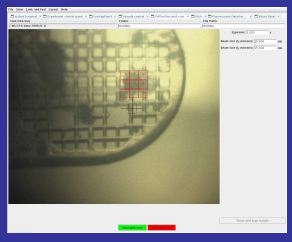




User operation

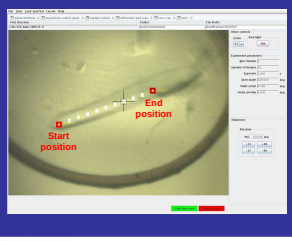
Experimental Control

Users use the Generic Data Acquisition (GDA) graphical interface to run their experiments in their entirety. This includes upload of sample data from ISPyB, sample centering, interface to robot, and initiation of experiment.
Through GDA users have full control of the beamline, with changes in beamsize at the sample and energy changes easily accessible. In addition tools are being developed to allow users to exploit the opportunities made available by a microfocus beamline.



Grid Scan

This tool allows a search grid to be stretched and dragged over loop/region of interest.
- Beam can be focused/defocused for fine/coarse scan
- Search for small crystals or more perfect regions of larger crystals



Line Scan

Start and end points for data collection are defined on the crystal via the gui allowing helical data collection.
- Unexposed crystal is continually introduced into the beam during data collection

http://www.diamond.ac.uk/I24

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